Systematics of *Fejervarya limnocharis* (Gravenhorst, 1829) (Amphibia, Anura, Ranidae) and related species. 2. Morphological and molecular variation in frogs from the Greater Sunda Islands (Sumatra, Java, Borneo) with the definition of two species

Michael VEITH*, Joachim KOSUCH*, Annemarie OHLER** & Alain DUBOIS**

* Institut für Zoologie, Abt. Ökologie, Johannes Gutenberg-Universität, Saarstr. 21, 55099 Mainz, Germany

** Laboratoire des Reptiles et Amphibiens, Muséum national d'Histoire naturelle, 25 rue Cuvier, 75005 Paris, France

Frogs of the species *Fejervarya limnocharis* and related species are among the most common in Southeast Asia. We studied 52 individuals from eight populations of the Great Sunda Islands by means of allozyme electrophoresis, mtDNA sequencing and morphometry. Patterns of variation of all characters among populations were congruent in separating one Javanese population from all other populations (Java, Sumatra, Borneo) as a distinct species. The frequencies of four distinct mid-dorsal stripe showed no distinct geographic pattern. Morphometric analysis unambiguously permits assignment of the name *Rana limnocharis* Gravenhorst, 1829 to the widespread form that occurs in Java, Sumatra and Borneo. The two taxa recognised in this study occur in sympatry in Java. As the taxon known only from Java lacks a name, we describe and name it.

ABBREVIATIONS

FMNH	Field Museum of Natural History, Chicago, USA.
MNHN	Muséum National d'Histoire Naturelle, Paris, France.
RMNH	Nationaal Natuurhistorisch Museum, Leiden, Netherlands.
ZFMK	Zoologisches Forschungsinstitut und Museum Alexander Koenig, Bonn, Germany.

Introduction

Description of existing biodiversity is one of the main goals of conservation biology. Especially in the tropics very little is known about the real number of species and our knowledge on intraspecific variability of tropical animal and plant species is even lower. The Amphibia of most parts of the Oriental faunal region have been poorly studied until now (Inger, 1999). This holds especially for the Greater Sunda Islands, although two reviews of its batrachofauna were recently published (Manthey & Grossmann, 1997; Inger & Stuebing, 1997). Within the last decades, many new species have been described from these islands based on morphological traits (e.g., Inger, 1989; Inger & Gritis, 1983; Inger & Stuebing, 1991, 1997). However, the few biochemical studies on Southeast Asian anurans have made apparent that a great amount of cryptic variation is present within species (e.g., Inger et al., 1974; Donnellan et al., 1989; Kosuch et al., 1997; Toda et al., 1998*a*).

The ranid genus *Fejervarya* Bolkay, 1915 is distributed throughout Southeast Asia (Dubois & Ohler, 2000). These species show substantial morphological and colour variation over their distribution range. In Nepal, studies of mating calls showed the co-existence of four species (Dubois, 1975). In their study on genetic divergence among Southeast Asian *Rana limnocharis,* Toda et al. (1997, 1998*a-b*) discovered genetic divergence and sympatric cryptic species in this group without morphological differentiation.

The present study focuses on the variation within frogs known as *Fejervarya limnocharis* (Gravenhorst, 1829) in the Greater Sunda Islands, a group of frogs that are known to be abundant in areas of intensive human activities (Inger, 1966; Inger & Stuebing, 1989; Manthey & Grossmann, 1997). To describe the pattern of variation in this common frog group we compared the within- and between-island variability of molecular and morphological traits.

The nomenclatural situation in this group of frogs was presented in detail in part 1 of this series of papers (Dubois & Ohler, 2000). Generic classification follows Dubois (1992), slightly modified by phylogenetic data of DNA analysis (Marmayou et al., 2000). In order to formalise the systematic position of the different forms of *Fejervarya* found in our genetic analysis, we linked molecular data to morphometric data. This allows us to cross-reference old names based on museum specimens with results of modern molecular population studies.

Material and methods

Frogs were collected from six natural populations or bought from local fish markets of Java (J), Sumatra (S) and Kalimantan (K), the latter being the Indonesian part of Borneo (fig. 1): J1 (4 specimens, FMNH 256721-256724), swampy meadow along the road between Bogor and Parung; J2 (11 specimens, FMNH 256725-256733, MNHN 1997.4916, ZMFK 68867), paddy field at Chianjur; K1 (2 specimens, FMNH 256734-256735), fish market at Pontianak; K3 (12 specimens, FMNH 256736-256747), paddy field at the village Desa Lape; S1 (13 specimens, FMNH 256749-256761), fish market at Medan; S2 (2 specimens, FMNH 256762-256763), paddy field and small brook at village Sidikalang; S5 (5 specimens, FMNH 256764-

Fig. 1. – Sample localities of *Fejervarya* on the Great Sunda Islands, Indonesia (* refers to the sample locality 3/4 of TODA et al., 1998*a*).

256768), frogs collected by inhabitants at a small village about 10 km SE Tapaktuan; S6 (3 specimens, FMNH 256769-256771), frogs collected by inhabitants of the village Desa Seleukat near Tapaktuan. In the molecular analyses we used the ranids *Fejervarya cancrivora* (Gravenhorst, 1829) (Kalimantan, Borneo), *Rana temporaria* Linnaeus, 1758 (Germany) and *Rana catesbeiana* Shaw, 1802 (from a frog farm at Java) as outgroups.

A specimen from the collection of Heinrich Kuhl from Java (RMNH 4287), designated as neotype of *Rana limnocharis* Gravenhorst, 1829 by DUBOIS & OHLER (2000), was included in a discriminant analysis for nomenclatural decisions.

Sex of all specimens was determined either by presence of secondary sexual characters or by observation of gonads through small lateral or ventral incision on the frogs (most of the frogs were ventrally opened because of tissue dissection in the field).

ALLOZYME ELECTROPHORESIS

Pieces of fresh muscle and liver were dissected and stored in liquid nitrogen. Frogs were preserved for determination and morphometry in 70 % alcohol after pre-fixation in 10 % formaldehyde.

Allozyme electrophoresis was carried out on muscle and liver homogenates on cellulose acetate gels (RICHARDSON et al., 1986). Twelve enzyme systems providing information on 15 presumptive gene loci were stained according to HEBERT & BEATON (1986). Gels were run in three different buffer systems: tris-glycine, pH 8.5 [*acon-1* and *-2* (E.C. 4.1.1.3), *fum* (E.C. 4.2.1.2), *aat-1* and *-2* (E.C. 2.6.1.1), *mdh* (E.C. 1.1.1.37), $pep_{Gly\text{-}Leu}$ (E.C. 3.4.11), *pgm* (E.C. 5.4.2.2) and *tpi* (E.C. 5.3.1.1)], tris-citrate, pH 7.0 [*idh-1* and *-2* (E.C. 1.1.1.42), *me* (E.C. 1.1.1.40) and *mpi* (E.C. 5.3.1.8)] and phosphate, pH 8.0 [*ldh* (E.C. 1.1.1.27) and *pk* (E.C. 2.7.1.40)]. Average expected heterozygosity (H_e) and mean number of polymorphic loci (P) were calculated as population characteristics using the computer program G-STAT (SIGISMUND, 1993). NEI's (1972) standard genetic distance estimates and UPGMA cluster analysis were calculated using the program NTSYS (ROHLF, 1990).

DNA-SEQUENCING

DNA was extracted from frozen muscle tissue using the standard phenol-chloroform protocol of SAMBROOK et al. (1989). Double-stranded PCR amplification was performed in 50 µl reactions containing 1 unit of Taq polymerase, 5 µl of 10 × reaction buffer (Boehringer), 250 µmol each of dGTP, dATP, dTTP and dCTP, 20 pmol each of light- and heavy-strand primers and 1 µl of mtDNA extract. The primers 16Sa-L and 16Sb-H (KOCHER et al., 1989) amplified a double-stranded DNA segment of 560 bp of the mitochondrial 16S ribosomal RNA gene. Thermal cycling was carried out in a programmable heating block (Perkin Elmer Gene Amp PCR System 9600) for 35 cycles (initial denaturation step of 90 s at 94°C, denaturation for 45 s at 94°C, primer annealing for 45 s at 55°C and extension for 90 s at 72°C).

PCR products were purified with a PCR purification kit (QuiaQuickspin) and directly sequenced with a sequenase kit (Amersham) using only primer 16Sa-L. The products of the sequencing reaction were resolved by the automatic sequencer 377 of Applied Biosystems.

After sequence alignment with CLUSTAL-W (HIGGINS & SHARP, 1993), the alignment was adjusted manually. The aligned 390 bp sequence corresponds to nucleotides 4039-4429 in the *Xenopus laevis* mitochondrial genome (ROE et al., 1985). Phylogenetic relationships were determined using maximum parsimony (PAUP, version 3.1.1; SWOFFORD, 1993) and maximum likelihood approaches (PHYLIP, version 3.5c; FELSENSTEIN, 1993). For maximum parsimony analysis, 1000 bootstrap replicates were run to test for confidence in the topology of the phylogenetic trees (FELSENSTEIN, 1985).

Sequences have the GenBank accession numbers AF346810-AF346811, and the EMBL accession numbers AJ292014-AJ292023.

MORPHOMETRY

Twenty-seven measurements of 39 adult specimens were taken with a slide caliper to 0.1 mm precision or for measurements smaller than 5 mm with an ocular micrometer to the nearest 0.01 mm (app. 1). To control for isometry, all measurements were divided by snout-vent length (SVL), expressed as per thousands of SVL, or transformed into the Neperian logarithm. Male and female specimens were grouped after their homogeneity was established

(OHLER, 1996). For morphometrical analyses we used SPSS statistical programs for personal computers (NORUSIS, 1992)

Morphological analysis should test if genetically close populations from different islands are also morphologically homogenous and, conversely, if the two genetically differentiated species can be distinguished by their morphology. Non-parametric Kruskal Wallis test was performed to measure morphological variation in *Fejervarya* specimens of different islands. Differentiation between genetically distinct samples was tested using non-parametric Mann-Whitney *U* test.

Discriminant analysis

Groups obtained by the molecular analyses were subjected to a discriminant analysis (NORUSIS, 1992) The measurement data for the female neotype of *Rana limnocharis* were incorporated into the analysis after the production of the original discriminant function model based on all other data, following examples of nomenclatural clarification using this approach (HEYER, 1994; OHLER, 1999; OHLER & DUBOIS, 1999).

Colour patterns

Frogs of the genus *Fejervarya* are known to frequently have mid-dorsal stripes, which are an interesting character for evolutionary biology studies (see e.g MILSTEAD et al., 1974; DUBOIS, 1980). Heritability studies by MORIWAKI (1953) and MOHANTY & DUTTA (1999) suggested that presence or absence of a dorsal line in *Fejervarya* is coded by two alleles of a single locus. The allele for the striped phenotype appears to be dominant over that for the unstriped one. However, MORIWAKI (1953) and others (e.g., SHIBATA, 1988) only discriminated between two phenotypes (striped and unstriped). DUBOIS (1977) and MOHANTY & DUTTA (1999) distinguished between three phenotypes, a pattern showing no line at all, a pattern showing a fine line and a third pattern showing a wide stripe The patterns are not distributed equally in the four different species of *Fejervarya* from Nepal which can be distinguished by their mating calls, whereas *F. teraiensis* (Dubois, 1984) and *F. pierreti* (Dubois, 1975) show all three different patterns, *F. syhadrensis* (Annandale, 1919) does not show the wide stripe phenotype, and all *F. nepalensis* (Dubois, 1975) specimens have mid-dorsal stripes (DUBOIS, 1977)

Four mid-dorsal patterns can be recognised in the *Fejervarya* specimens that we studied (fig. 2): (1) no mid-dorsal line or stripe, (2) a fine clear mid-dorsal line alone present, (3) a wide mid-dorsal stripe alone present, (4) both a fine line and a wide stripe present, superimposed. Presence and absence of both kinds of lines as well as frequency of combination of the two lines and their distribution among populations were noted, and their pattern of variation was studied using Kruskal-Wallis test.

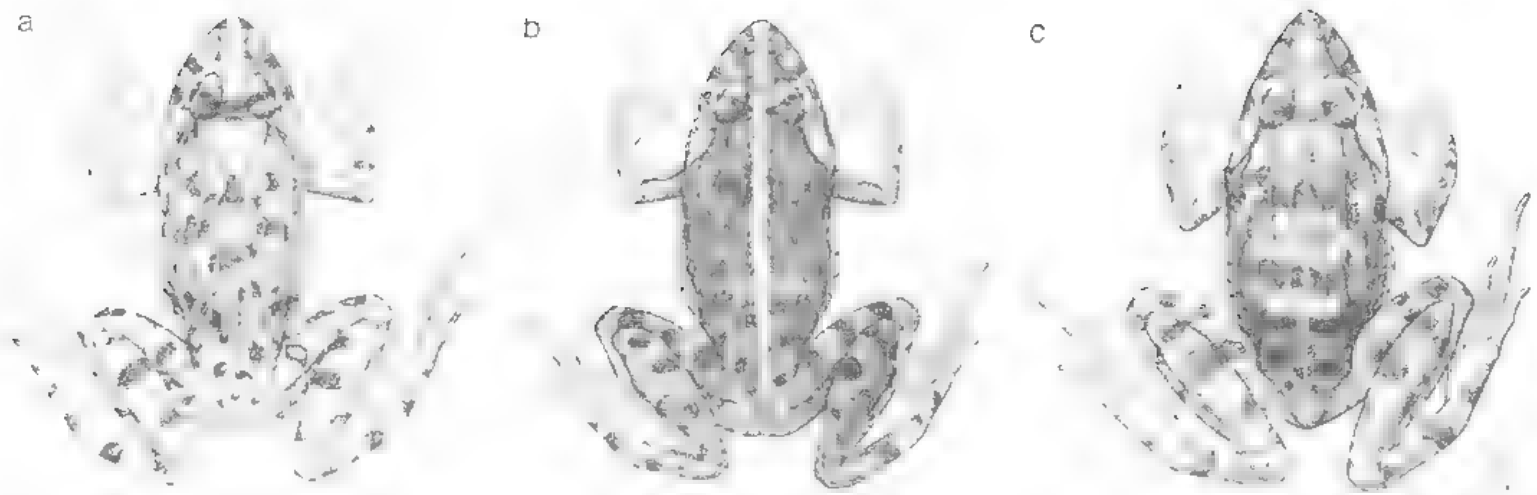

Fig. 2 — (a) Holotype of *Fejervarya iskandari* MNHN 1997.4916, from Chianjur, Java, phenotype no line/no stripe, 16S haplotype J2-C, (b) *Fejervarya limnocharis* from Desa Lape, Borneo, phenotype line/stripe, 16S haplotype K3-A, (c) *Fejervarya limnocharis* from Tapaktuan 2, Sumatra, phenotype line/no stripe, 16S haplotype S5-A. Drawings by Käthe Rehbinder, Mainz.

RESULTS

ALLOZYME VARIATION

Of the 15 allozyme loci studied, five were monomorphic (*fum*, *idh-1*, *idh-2*, *me*, *pk*) within and between the *Fejervarya* samples. *Acon-1*, *acon-2* and *ldh* were monomorphic within populations, but exhibited a fixed allelic difference in population J2 relative to all other populations (app. 2). Between populations J1 and J2 further allelic differences appeared to be fixed at the loci *aat-2*, *mpi* and *pep*. Thus, these two geographically closely related populations did not share alleles at six out of 15 loci.

Intra-populational genetic variability (H_e) was lowest in the Sumatran samples with the exception of sample S1 from the Medan fish market. This was the only sample that showed significant genotype deviations from Hardy-Weinberg expectation at two loci (*mpi* and *pep*; $P < 0.05$) due to a lack of heterozygous specimens, indicating a mix-up of different populations. Sample S5 was completely monomorphic. Three to six loci were polymorphic in the Javanese and Borneo samples, even though sample sizes were in some cases lower than for S5.

NEI's (1972) standard genetic distance between populations ranged from 0.004 between S1 and S2 and 0.337 between J2 and S5 (tab. 1). Population J2 was the genetically most distinct. It was separated even from the geographically close population J1 by a relatively high genetic distance estimate of 0.316. The Borneo and Sumatra populations formed distinct clusters in the UPGMA phenogram (fig. 3).

MTDNA SEQUENCE DIVERGENCE

We sequenced 390 bp of the mitochondrial 16S rRNA gene from 52 individuals of *Fejervarya* and from the three outgroup species. One hundred twenty seven bp positions were variable. Samples of *Fejervarya* comprised nine different haplotypes (tab. 2) with 60 sites being variable among them. Fifty-two of these variable sites were parsimonious informative, 49 of which supported the monophyly of either of the two main haplotype lineages.

The average substitution rate between the haplotypes of population J2 and the remaining populations from Java, Sumatra and Borneo was 0.135. This is only about 0.02 less than between either of the two *Fejervarya* forms and *F. cancrivora*, thus suggesting the differentiation of J2 from the other *Fejervarya limnocharis* populations as distinct species.

The maximum parsimony and maximum likelihood trees produced exactly the same topology with two rather homogeneous but well differentiated groups of *Fejervarya* mitochondrial 16S haplotypes (tab. 3). Bootstrap support in the maximum parsimony tree was 100 % for both clades (fig. 4) which we subsequently name J1 and J2. No further significant topology was found within the two clades.

Table 1 – NEI's (1972) standard genetic distance estimates between 9 samples of *Fejervarya* from Java (J), Kalimantan (K) and Sumatra (S) See text (*Material and methods*) for details on localities Sample Kc belongs to the species *Fejervarya cancrivora*

	J1	J2	K1	K3	S1	S2	S5	S6
J2	0 316							
K1	0 188	0 282						
K3	0 169	0 298	0 026					
S1	0 102	0 319	0 071	0 034				
S2	0 182	0 324	0 122	0 059	0 040			
S5	0 195	0 337	0 154	0 089	0 054	0 004		
S6	0 178	0 320	0 097	0 037	0 034	0 004	0 017	
Kc	1 198	1 088	0 968	0 974	1 021	0 957	0 970	0 953

Table 2 – Variable bp positions of a partial sequence of the mitochondrial 16S-rRNA gene of nine *Fejervarya* and three outgroup taxa haplotypes.

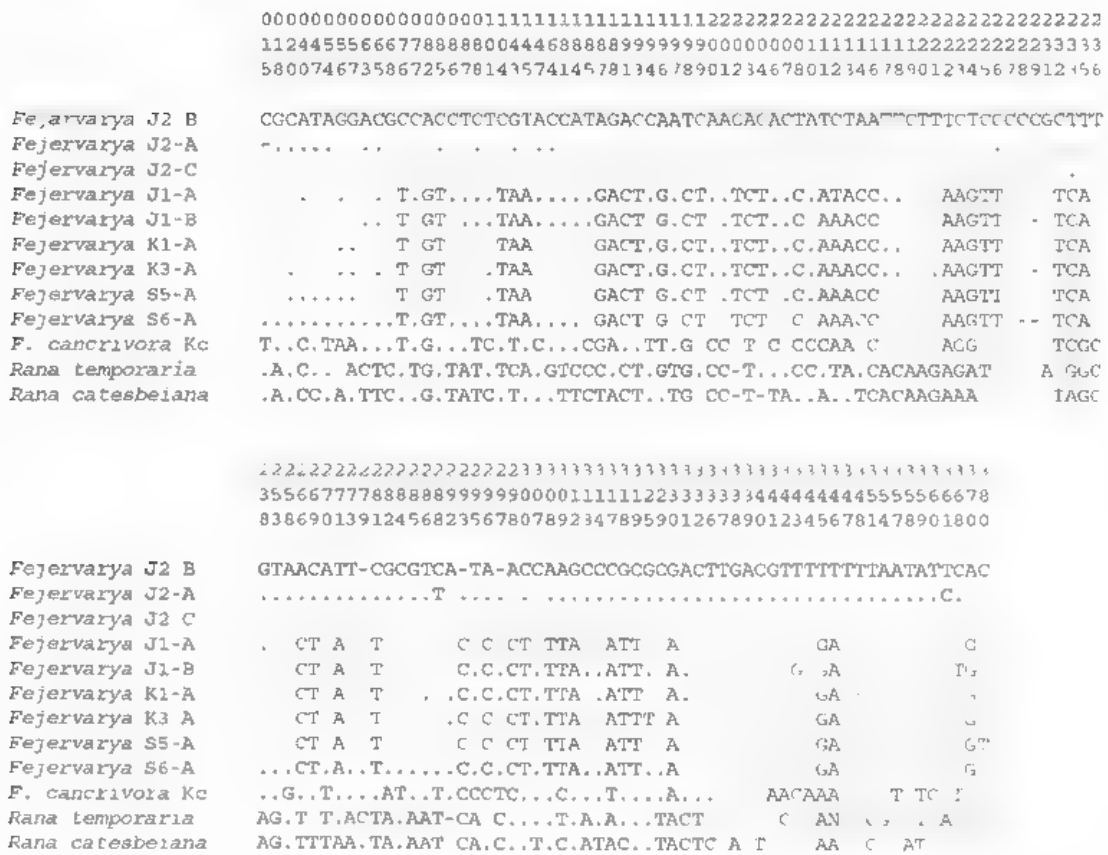

Table 3 – Average substitution rate between samples of the two *Fejervarya* lineages J1 and J2 and three outgroup taxa, transitions and transversions were weighted equally, gaps treated as fifth base

	J1 lineage	J2 lineage	*F. cancrivora*	*R. temporaria*
J1 lineage (J/K/S)	0.006 ± 0 004			
J2 lineage	0.135 ± 0.004	0 006 ± 0.003		
F. cancrivora	0 160 ± 0 001	0 155 ± 0 001	-	
R. temporaria	0 219 ± 0 002	0 229 ± 0 001	0.210	-
R. catesbeiana	0 209 ± 0 002	0 212 ± 0 002	0 192	0 112

MORPHOMETRIC VARIATION

For all measurements and ratios, the mean, standard deviation and minimum and maximum values are given for adult specimens of *Fejervarya* separating samples by sex and geographical origin (app. 3) Comparisons of adult individuals using the Kruskal-Wallis test (tab. 4) show significant differences in the frogs from the three islands in the ratios which all concern head shape (head length, distance between anterior border of eyes, distance between posterior border of eyes, internarial distance) *Fejervarya* from Java have significantly shorter heads and more pointed snouts than the frogs from Sumatra and Borneo. Frogs from Sumatra are significantly distinct in having a greater distance between posterior border of eyes (app. 3, tab. 4).

Dorsal pattern

In the populations we studied, 32 1 % of the specimens have no mid-dorsal stripe, a fine mid-dorsal line is present in 57.6 % of all specimens examined, a wide stripe in 30 5 % of the specimens, and the combined fine-wide striped phenotype was observed in 15 3 % of the specimens (tab. 5).

More males lack a mid-dorsal line and more females have a combination of both phenotypes (chi-square test, $\chi^2 = 23\ 0181$, $df = 3$, $P < 0\ 001$). Neither males nor females differ in a significant manner from the overall distribution of dorsal pattern (chi-square test: males, $\chi^2 = 5.7635$, $df = 3$, $P > 0\ 05$; females, $\chi^2 = 5\ 1466$, $df = 3$, $P > 0\ 05$).

The different populations can be put in three groups a series of populations showing no wide stripe phenotypes (group 1 in table 5), a population from Sumatra showing no narrow line pattern (group 3 in table 5), and a group of populations showing all four patterns (group 2 in table 5) Statistical test shows heterogeneity of phenotype distribution for the wide stripe pattern between populations studied (Kruskal-Wallis test, $\chi^2 = 22\ 38$, $df = 7$, $P < 0\ 01$) Distribution between populations for narrow line pattern is not statistically heterogeneous (Kruskal-Wallis test: $\chi^2 = 12\ 05$, $df = 7$, $P > 0\ 05$) The population from Chianjur is in the

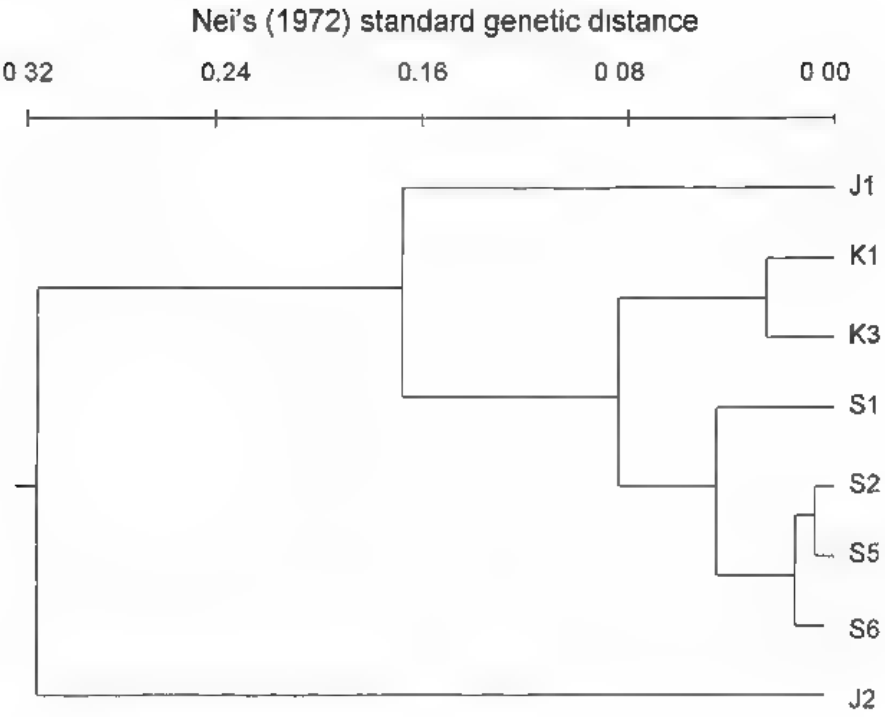

Fig 3 – UPGMA phenogram of Nei's (1972) standard genetic distances between the *Fejervarya* samples (allozyme data), the tree is rooted by the outgroup *Fejervarya cancrivora*; the cophenetic value is 0 99

same dorsal pattern group as the population from Desa Lape (Borneo) and the population from Medan (Sumatra) The two Javanese populations are not in the same dorsal pattern group

In conclusion, populations of *Fejervarya* from the Sunda Islands show statistically significant variation of dorsal colour patterns, but this variation is not congruent with allozyme or mtDNA variation as shown in this study.

To which population does the name Rana limnocharis *Gravenhorst, 1829 apply?*

The name-bearing type provides the objective standard of reference by which the application of the name it bears is determined (Anonymous, 1999: Article 61.a) Generally, taxa have been described using morphological methods, and new techniques are rarely applicable to dead museum specimens Systematics is, however, a largely historical science, as new results are added to previous knowledge One scope of modern science therefore is to define methods to find quantifiable links between historical museum specimens (especially type-specimens) and new material studied by modern methods.

Multivariate statistics have been used to refer single type specimens to biologically defined populations (Heyer, 1994, Ohler, 1996, 1999, Ohler & Dubois,1999) This also applies to the case of the two *Fejervarya* lineages here defined by allozyme and DNA data.

The historical specimen of Heinrich Kuhl, who first collected the species *Rana limnocharis*, was a judicious choice for a neotype (see Dubois & Ohler, 2000) In order to find out to

Table 4 – Comparison by Kruskal Wallis test of snout-vent length (SVL) and of ratios of measurements in adult specimens of *Fejervarya* from different origins. For each sample, minimum and maximum values, mean and standard deviation are given. *df*, degree of freedom, *n* sample size, *P*, probability, *, significance level $P \leq 0.05$

	Java *n* = 3	Sumatra *n* = 17	Kalimantan *n* = 14	Kruskall-Wallis test *df* = 2
FL / SVL	96 – 117 108 ± 10.6	97 – 129 112 ± 8.59	109 – 126 115 ± 4.60	$\chi^2 = 2.783$ *P* = 0.249 *ns*
FN / SVL	86 – 91 88 ± 2.55	80 – 101 91 ± 5.45	81 – 95 89 ± 4.70	$\chi^2 = 2.010$ *P* = 0.366 *ns*
FLL / SVL	196 – 221 209 ± 12.6	191 – 227 215 ± 9.12	207 – 230 221 ± 6.80	$\chi^2 = 5.259$ *P* = 0.072 *ns*
FOL / SVL	496 – 554 523 ± 29.2	490 – 563 533 ± 20.4	465 – 560 530 ± 24.1	$\chi^2 = 0.576$ *P* = 0.750 *ns*
FTL / SVL	303 – 324 316 ± 11.6	292 – 338 319 ± 14.4	273 – 344 319 ± 16.8	$\chi^2 = 0.407$ *P* = 0.816 *ns*
HAL / SVL	176 – 221 199 ± 22.5	173 – 219 197 ± 13.2	180 – 223 203 ± 12.0	$\chi^2 = 1.601$ *P* = 0.449 *ns*
HL / SVL	329 – 375 356 ± 24.1	369 – 448 388 ± 17.8	363 – 416 391 ± 14.5	$\chi^2 = 6.849$ *P* = 0.033 *
HW / SVL	342 – 360 350 ± 9.34	332 – 365 347 ± 10.3	319 – 367 348 ± 13.3	$\chi^2 = 0.268$ *P* = 0.875 *ns*
IBE / SVL	210 – 246 232 ± 18.8	208 – 249 225 ± 11.5	217 – 271 238 ± 14.8	$\chi^2 = 6.668$ *P* = 0.036*
IFE / SVL	129 – 145 138 ± 8.60	135 – 162 147 ± 8.87	141 – 173 156 ± 10.1	$\chi^2 = 8.680$ *P* = 0.013*
IMT / SVL	44 – 56 50 ± 6.26	45 – 57 51 ± 3.21	46 – 57 51 ± 3.49	$\chi^2 = 0.542$ *P* = 0.762
IN / SVL	66 – 69 68 ± 1.58	68 – 79 73 ± 3.13	66 – 85 75 ± 4.69	$\chi^2 = 7.739$ *P* = 0.021 *
ITL / SVL	96 – 126 108 ± 15.4	103 – 123 116 ± 4.95	108 – 125 118 ± 5.38	$\chi^2 = 2.148$ *P* = 0.342
MBE / SVL	140 – 154 145 ± 8.1	137 – 171 149 ± 11.0	135 – 193 158 ± 17.3	$\chi^2 = 2.840$ *P* = 0.242 *ns*
MFE / SVL	221 – 255 237 ± 17.5	226 – 294 249 ± 16.0	229 – 279 252 ± 14.7	$\chi^2 = 1.746$ *P* = 0.418 *ns*
MN / SVL	306 – 326 314 ± 10.7	310 – 393 330 ± 19.2	304 – 373 335 ± 16.2	$\chi^2 = 5.339$ *P* = 0.069 *ns*
SVL	44.4 – 55.2 48.4 ± 5.91	32.6 – 59.0 48.6 ± 8.36	36.2 – 54.2 46.1 ± 6.06	$\chi^2 = 1.458$ *P* = 0.482 *ns*
TiL / SVL	114 – 130 123 ± 7.97	102 – 130 118 ± 6.76	105 – 135 123 ± 7.84	$\chi^2 = 4.216$ *P* = 0.121 *ns*
TL / SVL	489 – 532 504 ± 23.9	475 – 537 514 ± 17.1	465 – 534 508 ± 16.9	$\chi^2 = 1.772$ *P* = 0.412 *ns*
TYD / SVL	63 – 66 65 ± 1.86	58 – 69 63 ± 3.33	59 – 73 66 ± 4.05	$\chi^2 = 5.454$ *P* = 0.065 *ns*
TYE / SVL	35 – 40 37 ± 2.45	32 – 44 39 ± 3.32	27 – 49 40 ± 4.74	$\chi^2 = 1.408$ *P* = 0.495
WEE / SVL	73 – 102 83 ± 16.5	63 – 92 77 ± 8.26	52 – 97 78 ± 12.2	$\chi^2 = 0.207$ *P* = 0.902 *ns*
WI / SVL	75 – 87 82 ± 5.9	65 – 88 77 ± 7.39	63 – 92 79 ± 8.78	$\chi^2 = 1.083$ *P* = 0.582
WII / SVL	55 – 79 64 ± 12.7	40 – 75 60 ± 8.97	46 – 79 60 ± 9.49	$\chi^2 = 0.222$ *P* = 0.895 *ns*
WIF / SVL	86 – 108 96 ± 11.2	74 – 100 87 ± 8.69	77 – 104 90 ± 8.07	$\chi^2 = 2.713$ *P* = 0.258 *ns*

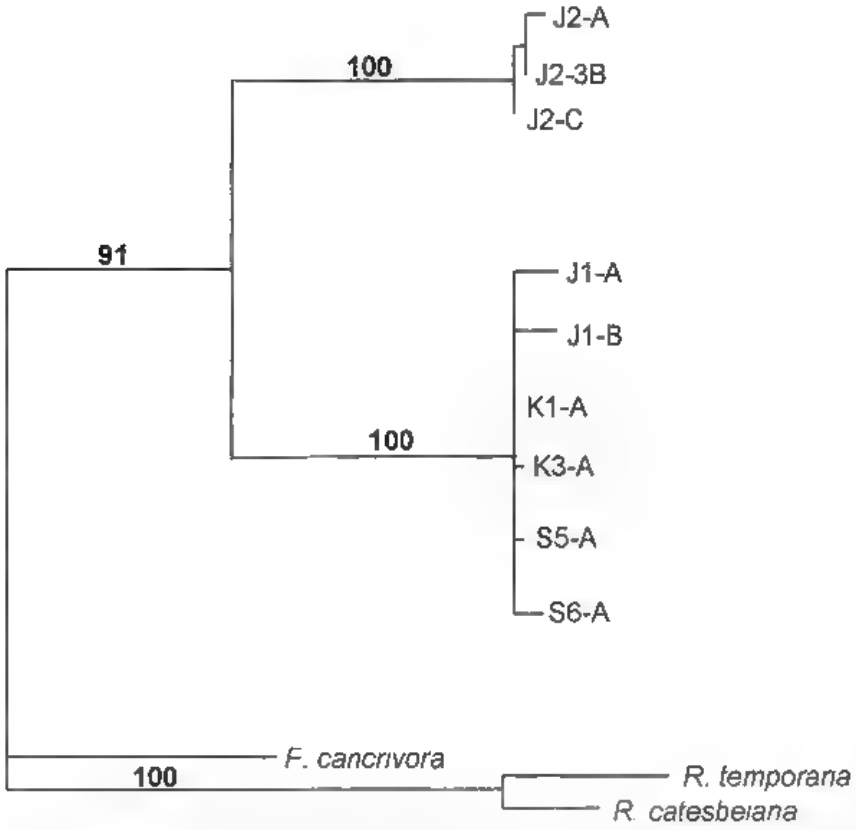

Fig. 4 Maximum parsimony tree of 390 bp of the mitochondrial 16S rRNA gene of nine *Fejervarya* haplotypes. Tree length is 202 steps (one shortest tree only), bootstrap values > 50 % for 1000 replicates are indicated

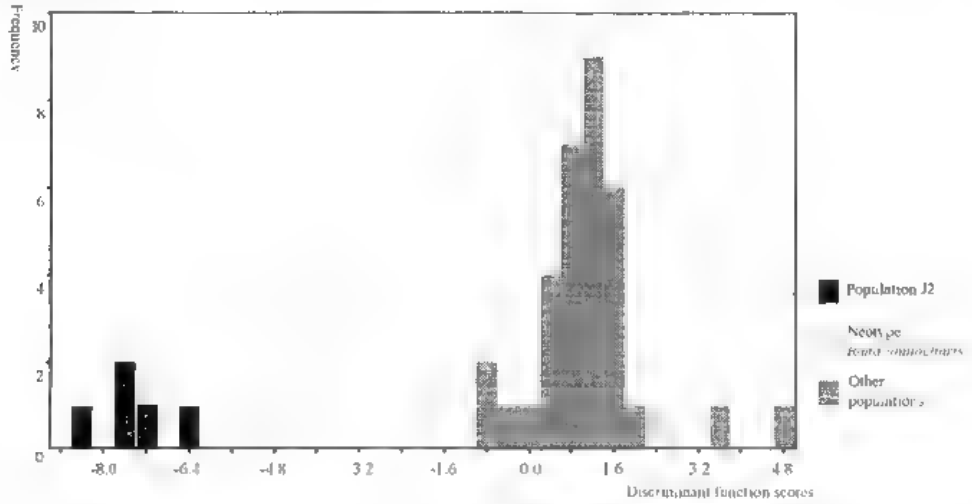

Fig. 5 Stacked histogram of discriminant function for specimens of population J2 and other populations of *Fejervarya* from Sunda Islands. The neotype RMNH 4287 of *Rana limnocharis* Gravenhorst, 1829 was included into this analysis without a priori group membership

Table 5 Coloration pattern of dorsum in *Fejervarya* from Sunda Islands Frequency distribution of phenotypes of mid-dorsal line and alleles supposed present in populations studied n, allele wild narrow line, N, allele narrow line, w, allele wild wide line, W, allele wide line Proposed phenotypical groups 1, line absent or narrow line present (wide line never observed), 2, line absent or fine and or wide line present (all 4 phenotypes observed), 3, line absent or wide line present (fine line never observed)

Sample (sample size)	Line absent	Fine line	Wide line	Fine and wide line	Alleles inferred to be present in genotype	Proposed group
J1 Bogor (4)	2	2	0	0	n, N / w	1
S2 Sidikalang (2)	1	1	0	0	n, N / w	1
S5 Tapaktuan (5)	2	3	0	0	n, N / w	1
S6 Desa Seleutkat (3)	2	1	0	0	n, N / w	1
S1 Medan (13)	1	10	0	2	n, N / w, W	2
J2 Chianjur (11)	5	3	1	2	n, N / w, W	2
K3 Desa Lape (13)	2	2	6	3	n, N / w, W	2
K1 Pontianak (2)	0	0	2	0	n / w, W	3

which genetically defined lineage (population J2 or the other populations from Sunda Islands) this name applies, a discriminant analysis was performed and subsequently applied to the neotype of *Rana limnocharis*. The analysis clearly classes the neotype with lineage J1 and not with the specimens from population J2 (tab. 6, fig. 5). This indicates that the name *Rana limnocharis* Gravenhorst, 1829 should apply to the widely distributed taxon occurring in Java, Sumatra and Borneo, and that a new name should be created for the species from population J2. As no other name is available for this taxon (see DUBOIS & OHLER, 2000), we describe it as a new species. The morphological comparison of adult males of the new species and *Fejervarya limnocharis* is summarised in table 7.

Fejervarya iskandari sp. nov.

Diagnosis Medium sized *Fejervarya* with a relatively wide head and interorbital distance, small tympanum, short eye length, short forearms, short inner toes and small inner metatarsal tubercles.

Description of holotype MNHN 1997.4916, adult male (fig. 2a), from paddy field at Chianjur (Java) (6°12'S, 107°08'E).

(A) Size and general aspect (1) Specimen of rather small size (SVL 40.4 mm), body moderately stout.

Table 6 – Results of canonical discriminant analysis between genetically determinated specimens of *Fejervarya*

A Statistical significance

Eigenvalue	Canonical correlation	Wilks Lambda	Chi-square	Degrees of freedom	*P*
8.7871	0.9475	0.102175	52.465	26	0.0016

B Standardised canonical discriminant function coefficients

Morphometric character	Function 1
EL	1.86131
EN	– 3.16796
FLL	1.38762
FOL	0.52334
FTL	– 6.44177
HAL	– 0.01355
HL	– 11.18890
HW	– 1.91388
IBE	– 3.85940
IFE	2.55580
IN	1.88979
ITI	– 0.99019
IUE	– 0.29879
MBE	– 3.25966
MFE	5.56794
MN	4.88480
SVL	42.020
TFL	1.21313
TL	7.26577
TYD	2.31338
TYL	1.93537
UEW	0.69464
WFI	0.54972
WI	1.86954
WII	– 0.75687
WIT	– 0.96165

C Classification success

Actual group	Predicted group	
	F. tiskandari	*F. limnocharis*
Fejervarya tiskandari	5 (100 %)	0
Fejervarya limnocharis	0	33 (100 %)
Neotype of *Rana limnocharis* (ungrouped)	0	1 (100 %)

Table 7 Comparison of adult males of *Fejervarya iskandari* sp. nov and *Fejervarya limnocharis* (Gravenhorst, 1829) by Mann-Whitney *U* test *n*, sample size, *P*, probability, *U*, Mann-Whitney *U*, *ns*, significance level P > 0 05, **, significance level $P \leq 0\,01$

Variable	*F. iskandari* n = 5	*F. limnocharis* n = 7	Mann-Whitney *U* test
EL / SVL	106 ± 2.89 102 – 109	120 ± 6.87 112 – 129	*U* = 0 *P* = 0 0045 **
EN / SVL	94 ± 4.75 88 – 99	91 ± 6.81 82 – 101	*U* = 14 *P* = 0 5698 *ns*
FLL / SVL	209 ± 6.12 199 215	222 ± 4.36 218 – 230	*U* = 0 *P* = 0 0045**
FOL / SVL	524 ± 27 16 489 – 557	536 ± 12.62 522 – 557	*U* = 14 *P* = 0 5698 *ns*
FTL / SVL	323 ± 13 44 308 – 337	324 ± 8.37 315 – 337	*U* = 16 *P* = 0 8075 *ns*
HAL / SVL	212 ± 8.74 200 224	203 ± 15.74 179 – 223	*U* = 11 *P* = 0 2912 *ns*
HL / SVL	395 ± 17.18 374 – 416	397 ± 23.56 375 – 447	*U* = 17 *P* = 0 9353 *ns*
HW / SVL	358 ± 12 337 – 367	348 ± 5.96 340 – 354	*U* 7 *P* = 0.0882 *ns*
IBE / SVL	239 ± 5 54 232 – 245	244 ± 16.43 221 – 273	*U* = 13 *P* = 0 4649 *ns*
IFE / SVL	152 ± 10.67 141 – 162	157 ± 7 52 144 – 168	*U* = 14 *P* = 0 5698 *ns*
IMT / SVL	44 ± 3 19 43 – 48	50 ± 2.8 46 – 53	*U* 1 *P* = 0.0526 *ns*
IN / SVL	74 ± 1.68 72 – 76	76 ± 4.73 71 – 85	*U* = 15 *P* = 0.6847 *ns*
ITL / SVL	110 ± 10 1 96 – 124	119 ± 3.92 113 – 124	*U* 7 *P* = 0 0882 *ns*
IUE / SVL	54 ± 2.41 50 – 56	50 ± 3.78 46 – 55	*U* = 7 *P* = 0 0882 *ns*
MBE / SVL	163 ± 20.53 138 188	153 ± 14.13 134 171	*U* = 12 *P* = 0.3718 *ns*
MFE / SVL	255 ± 19 8 234 – 275	255 ± 19.62 237 – 294	*U* = 16 *P* = 0 8075 *ns*
MN / SVL	340 ± 19 07 318 – 360	339 ± 25 19 320 – 393	*U* = 16 *P* = 0 8075 *ns*
SVL	417 ± 11 37 404 – 427	398 ± 54 6. 326 – 467	*U* ,4 *P* = 0.5691 *ns*
TFL / SVL	124 ± 11 21 111 – 136	123 ± 4.9 118 – 132	*U* = 15 *P* = 0 6847 *ns*
TI SVL	494 ± 17 51 468 – 511	507 ± 12 76 488 523	*U* = 8 *P* = 0 1229 *ns*
TYD / SVL	67 ± 2.87 63 – 70	65 ± 5.34 59 – 73	*U* = 14 *P* = 0 5698 *ns*
TYE / SVL	33 ± 2 93 31 – 38	38 ± 4 29 32 43	*U* = 7 *P* = 0 0882 *ns*
UEW / SVL	83 ± 5 98 76 – 88	91 ± 4 67 87 – 100	*U* = 7 *P* = 0.0882 *ns*
WFE / SVL	81 ± 10 69 69 – 98	80 ± 11 63 61 – 97	17 0 9353 *n*
WI SVL	76 ± 12 99 64 – 96	81 ± 8.2 69 – 92	*U* = 12 *P* = 0 3718 *ns*
WII / SVL	62 12 11 47 – 80	61 10 8 46 – 79	*U* 15 *P* = 0 6847 *ns*
WTF / SVL	87 ± 12.03 76 – 106	92 ± 6.31 82 99	*U* = 10 *P* = 0 2232 *ns*

(B) Head. (2) Head of medium size, longer (HL 15.4 mm) than wide (HW 14.4 mm; MN 13.1 mm; MFE 9.5 mm; MBE 5.9 mm), convex. (3) Snout pointed, protruding, its length (SL 7.90 mm) much longer than horizontal diameter of eye (EL 4.34 mm). (4) Canthus rostralis rounded, loreal region very concave, vertical. (5) Interorbital space flat, smaller (IUE 2.27 mm) than upper eyelid (UEW 3.57 mm) and internarial distance (IN 2.92 mm); distance between front of eyes (IFE 5.7 mm) about half of distance between back of eyes (IBE 9.5 mm). (6) Nostrils oval with flap of skin laterally, closer to tip of snout (NS 3.55 mm) than to eye (EN 4.02 mm). (7) Pupil rounded. (8) Tympanum (TYD 2.72 mm) distinct, rounded, more than half of eye diameter; tympanum-eye distance (TYE 1.30 mm) about half its diameter. (9) Pineal ocellus present, between anterior border of eyes. (10) Vomerine ridge present, bearing few small teeth, between choanae, with an angle of 45° to body axis, less close to choanae than from each other, longer than distance between them. (11) Tongue large, rounded, emarginate. (12) Supratympanic fold distinct, from eye to shoulder. (13) Parotoid glands absent. (14) Cephalic ridges absent. (15) Co-ossified skin absent.

(C) Forelimbs. – (16) Arm short, rather thin (FLL 8.7 mm), as long as hand (HAL 8.7 mm), not enlarged. (17) Fingers I, III and IV long, finger II short, all thin (TFL 5.32 mm). (18) Relative length of fingers, shortest to longest: II < IV < I < III. (19) Tips of fingers pointed. (20) Finger II with dermal fringe; webbing absent. (21) Subarticular tubercles prominent, rounded, single, all present. (22) Prepollex oval, prominent; two oval, distinct palmar tubercles; supernumerary tubercles absent.

(D) Hindlimbs. – (23) Shank three times longer (TL 20.6 mm) than wide (TW 6.0 mm), longer than thigh (FL 18.8 mm), but shorter than distance from base of internal metatarsal tubercle to tip of toe IV (FOL 22.5 mm). (24) Toes long, thin; toe IV long (FTL 13.6 mm), more than one third of distance from base of tarsus to tip of toe IV (TFOL 29.6 mm). (25) Relative length of toes, shortest to longest: I < II < V < III < IV. (26) Tips of toes pointed. (27) Webbing moderate: I 1 – 2 II 1 – 2 III 1 – 2 2/3 IV 2 1/3 – 1 V (WTF 4.28 mm; WFF 3.95 mm; WI 3.89 mm; WII 3.24 mm; MTTF 9.7 mm; MTFF 10.3 mm; TFTF 9.5 mm; FFTF 10.0 mm). (28) Dermal fringe along toe V present, from tip of toe to base of metatarsus, well developed. (29) Subarticular tubercles prominent, oval, simple, all present. (30) Inner metatarsal tubercle long, very prominent, its length (IMT 1.94 mm) less than 2.5 times in length of toe I (ITL 4.60 mm). (31) Inner tarsal ridge well developed, along distal half of tarsus. (32) Outer metatarsal tubercle prominent, supernumerary tubercles absent, tarsal tubercle absent.

(E) Skin. – (33) Dorsal and lateral parts of head and body, snout smooth, between the eyes and side of head with few, flat glandular warts; back with glandular folds and glandular warts between them, flanks with glandular warts. (34) Latero-dorsal folds absent. (35) Dorsal parts of limbs: forelimbs smooth; shank, thigh and tarsus with glandular warts. (36) Ventral parts of head, body and limbs: throat, chest and belly smooth, thigh with glandular warts. (37) No macroglands.

(F) Coloration in alcohol. – (38) Dorsal and lateral parts of head and body: brown with blackish paired spots, shoulder spots indistinct, four brown spots on each side of upper lip. (39) Dorsal parts of limbs: forelimb, thigh, shank and foot light brown with dark bands, posterior part of thigh with brown and whitish marbling. (40) Ventral parts of head, body and limbs: chest, belly and thigh cream white; throat cream white with grey W-shaped pattern, margin of throat white with large brown spots.

(G) Male sexual characters. – (41) Nuptial spines in one single patch on prepollex and finger I; numerous, indistinct, cream coloured spines. (42) Vocal sacs as greyish, folded skin on the two sides of the throat; slit-like openings in rather anterior part of mouth floor. (43) Fine horny spinules on the anterior border of the throat.

(H) Part of the 16S rRNA sequence that corresponds to the nucleotides 3994-4554 of *Xenopus laevis* (ROE et al., 1985) – (44) (EMBL AJ292018) TCTTGTTTTTTCATAA GAGGTCCAGCCTGCCCAGTGACACAATTAAAGGCCGCGGTACCCTGACCGTG CGAAGGTAGCATAATAACTTGTTCTTTAAATGGGGACTAGCATGAACGGCAC CACGAAGGCCTCACTGTCTCCTTTTTCCAATCAGTGAAACTGATCTCCCCGTG AAGAAGCGGGGATGATAATATAAGACGAGAAGACCCCATGGAGCTTTAAACC CAATAAGCAACCCTAATCAACACAACTCATCTAAATTCTTTCTCCCCCTGCTTT TTGGTTTTAGGTTGGGGTGACCACGGAGTAAAACATATCCTCCACGACGTAC GGATTAACCCTTATCCAAGAGCCACCGCTCTAAGAATCGACAAATTGACGTTT TTTGATCCAATATATTGATCAACGAACCAAGTTACCCTGGGGATAACAGCGCA ATCCATTTTTAGAGCCCCTATCGACGAATGGGTTTACGACCTCGATGTTGGAT CAGGGTACCCAAGTGGTGCAGCCGCTACTAATGGTTTGTTTGTTCAACAATT AAAACCCTACGTGATCTGAGTTC.

Paratopotypes. – FMNH 256725-256733, 3 adult males, 1 adult female, 5 juvenile males; ZFMK 68867, adult male. Same collection data.

Etymology. – The new species is dedicated to Djoko Iskandar, herpetologist from Indonesia

DISCUSSION

Fejervarya iskandari sp. nov. and *Fejervarya limnocharis* (Gravenhorst, 1829) from Java clearly represent two genetically distinct lineages. Their Nei D values calculated from allozyme data (average 0.314) falls within the interspecific range known from other ranids (e.g., CASE, 1978; NISHIOKA & SUMIDA, 1990; MENSI et al., 1992; ARANO et al., 1993; BERLI et al., 1996; VEITH, 1996). However, the observed morphological variation between the genetically well differentiated lineages *F. iskandari* and *F. limnocharis* is slightly more pronounced than among members of the other populations, as estimated by the number of significantly distinct measurements. Therefore these two forms are clearly a pair of sibling species that are morphologically scarcely distinct from one another, but show substantial genetic differentiation. In addition, a comparison of our allozyme data with those of the samples 3 and 4 of TODA et al. (1998*a*) reveals that their samples fit almost perfectly with our analysis: in nine out of eleven loci which were analysed in both studies the results are identical, one locus shows similar allele frequencies and only in one locus the results are different. Discrepancies in the results may have been caused by using different protocols in the two laboratories (see VEITH, 1994 for further examples). Therefore the new species includes specimens from the Javanese populations of Chianjur and Malingping (sample 4 in TODA et al., 1998*a*). It is genetically well defined, but morphologically very similar to *F. limnocharis*. Distribution ranges of *F. iskandari* and *F. limnocharis* overlap at least between Malingping and Chianjur over a distance of ca. 130 km. Syntopy was shown for a paddy field near Malingping, Java (TODA et al., 1998*a*), confirming species status of *F. iskandari*.

Discriminant analysis of morphometric data resolves the nomenclatural relevance of the observed genetic divergence. The name *Rana limnocharis* Gravenhorst, 1829 applies to a taxon widely distributed in the Sunda Islands. As there is no indication of genetic or morphological differentiation of the frogs from Borneo island, the name *Rana wasl* Annandale, 1917 (based on a holotype from Kuching, Sarawak, Malaysia, on this island) is here considered a junior subjective synonym of *Rana limnocharis* (see DUBOIS & OHLER, 2000), but remains available for further systematic decisions, should Bornean *Fejervarya* prove heterogeneous.

RÉSUMÉ

Les grenouilles de l'espèce *Fejervarya limnocharis* et d'espèces voisines de celle-ci sont parmi les plus communes en Asie du Sud-Est. Nous avons étudié 52 spécimens de huit populations des Iles de la Sonde par les méthodes de l'électrophorèse d'allozymes, du séquençage d'ADN mitochondrial et de la morphometrie. Les patrons de variation de tous les caractères entre les populations se montrent congruents pour séparer une population de Java de toutes les autres populations (Java, Sumatra, Bornéo) et considérer qu'elle appartient à une espèce distincte. Les fréquences des quatre phénotypes observes concernant la présence et la largeur d'une ligne médio-dorsale dans les populations examinées ne permettent pas de dégager de claire corrélation avec les caractères moléculaires et morphométriques. Notre analyse morphométrique permet d'attribuer sans ambiguite le nom *Rana limnocharis* Gravenhorst, 1829 à l'espèce à vaste répartition qui se trouve à Java, Sumatra et Bornéo. Les deux taxa vivant en sympatrie, nous donnons un nom nouveau, *Fejervarya iskandari*, à la population de Chianjur, Java.

ACKNOWLEDGEMENTS

We wish to thank R. Feldmann for his help in the field and K. Rehbinder for her outstanding drawings. This study was supported by the "German Science Foundation (DFG)", grant Nr. SE 506/5-1 and by the "German Bundesamt für Naturschutz (BfN)", grant Nr. Vw-534 11-5/92. The morphometric work was part of the programme of MLSR 2586 "Systematique et evolution des vertebres tetrapodes". This is publication Nr. 01-36 of PPF "Faune et flore du sud est asiatique" (Nr. 00-35, see DEUVE & TIAN 2001).

LITERATURE CITED

ANONYMOUS. 1999. *International code of zoological nomenclature*. Fourth edition. London, International Trust for Zoological Nomenclature: i-xxix + 1-306.

ARANO, B., ESTEBAN, M. & HERRERO, P., 1993. Evolutionary divergence of the Iberian brown frogs. *Ann. Sci. nat. Zool. Paris*, **14**: 49-57.

BEERLI, P., HOTZ, H. & UZZELL, T., 1996. – Geologically dated sea barriers calibrate a protein clock for Aegean water frogs. *Evolution*, **50**: 1676-1687.

CASE, S.M., 1978. – Biochemical systematics of members of the genus *Rana* native to western North America. *Syst. Zool.*, **27**, 299-311.

DEUVE, T. & TIAN, M.-Y., 2001. *Zolmopatrobus nanlingensis* n. gen., n. sp., de la Chine méridionale, premier représentant d'une sous-tribu nouvelle parmi les Trechidae Patrobinae (Coleoptera, Caraboidea). *Bull. Soc. ent. France*, in press.

DONNELLAN, S. C., ADAMS, M. & APLIN, K. P., 1989. A biochemical and morphological study of *Rana* (Anura: Ranidae) from the Chimbu Province, Papua New Guinea. *Herpetologica*, **45**: 336-343.

DUBOIS, A., 1975. – Un nouveau complexe d'espèces jumelles distinguées par le chant: les grenouilles du Népal voisines de *Rana limnocharis* Boie (Amphibiens, Anoures). *C. r. Acad. Sci.*, (D), **281**, 1717-1720.

----- 1977. Les problèmes de l'espèce chez les amphibiens anoures. *Mém. Soc. zool. France*, **39**: 161-284.

----- 1980. Populations, polymorphisme et adaptation: quelques exemples chez les amphibiens anoures. *In*: R. BARBAULT, P. BLANDIN & J.-A. MEYER (ed.), *Recherches d'écologie théorique – Les stratégies adaptatives*, Paris, Maloine: 141-158.

----- 1992. Notes sur la classification des Ranidae (Amphibiens Anoures). *Bull. Soc. linn. Lyon*, **61**: 305-352.

DUBOIS, A. & OHLER, A., 2000. Systematics of *Fejervarya limnocharis* (Gravenhorst, 1829) (Amphibia, Anura, Ranidae) and related species. 1. Nomenclatural status and type-specimens of the nominal species *Rana limnocharis* Gravenhorst, 1829. *Alytes*, **18** (1-2): 15-50.

FELSENSTEIN, J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution*, **39**: 783-791.

----- 1993. *PHYLIP (Phylogenetic Inference Package) version 3.5c*. Seattle, Univ. of Washington, Dep. Genetics.

GRAVENHORST, J. L. C., 1829. *Delitiae Musei Zoologici Vratislaviensis. Fasciculus primus, continens Chelonios et Batrachiae*. Lipsiae, Sumptibus Leopoldi Vossii: i-xiv + 1-106, pl. 1-17.

HEBERT, P. D. N. & BEATON, M. J., 1986. – *Methodologies for allozyme analysis using cellulose acetate electrophoresis*. Beaumont, TX, Helena Laboratories: 1-31.

HEYER, W. R., 1994. Variation within the *Leptodactylus podicipinus-wagneri* complex of frogs (Amphibia: Leptodactylidae). *Smithsonian Contrib. Zool.*, **546**: i-iv+1-124.

HIGGINS, D. G. & SHARP, P. M., 1993. CLUSTAL W: a package for performing multiple sequence alignment on a microcomputer. *Cabios*, **5**: 151-153.

INGER, R. F., 1966. – The systematics and zoogeography of the Amphibia of Borneo. *Fieldiana, Zool.*, **52**: 1-402.

----- 1989. – Four new species of frogs from Borneo. *Malayan Nature J.*, **42**: 229-243.

----- 1999. – Distribution of amphibians in Southern Asia and adjacent islands. *In*: W. F. DUELLMAN (ed.), *Patterns of distribution of amphibians: A global perspective*. Baltimore & London, The John Hopkins University Press: 445-471.

INGER, R. F. & GRITIS, P. A., 1983. Variation in Bornean frogs of the *Ansonia spinulifer* species group, with description of two new species. *Fieldiana, Zool.*, **19**: 1-13.

INGER, R. F. & STUEBING, R. B., 1989. – *Frogs of Sabah*. Sabah Parks Publication Nr. **10**.

----- 1991. New species of frogs of the genus *Leptobrachella* Smith (Anura, Pelobatidae) with a key to the species from Borneo. *Raffles Bull. Zool.*, **39**: 99-103.

----- 1997. – *A field guide to the frogs of Borneo*. Borneo, Natural History Publications: 1-205.

INGER, R. F., VORIS, H. K. & VORIS, H. H., 1974. Genetic variation and population ecology of some Southeast Asian frogs of the genera *Bufo* and *Rana*. *Biochem. Genet.*, **12**: 121-145.

KOCHER, T. D., THOMAS, W. K., MEYER, A., EDWARDS, S. V., PAABO, S., VILLABLANCA, F. X. & WILSON, A. C., 1989. Dynamics of mitochondrial DNA evolution in mammals: amplification and sequencing with conserved primers. *Proc. nat. Acad. Sci. USA*, **86**: 6196-6200.

KOSUCH, J., OHLER, A. & VEITH, M., 1997. Genetic and morphological variation in *Limnonectes limnocharis* (Gravenhorst, 1829) from the Great Sunda Islands (Sumatra, Java, Borneo). *In*: Z. ROČEK & S. HART (ed.), *Abstracts of the Third World Congress of Herpetology 1997*: 249.

MANTHEY, U. & GROSSMANN, W., 1997. *Amphibien und Reptilien Südostasiens*. Münster, Natur und Tier: 1-512.

MARMAYOU, J., DUBOIS, A., OHLER, A , PASQUET, E & TILLIER, A , 2000 – Phylogenetic relationsh.ps in the Ranidae. Independent origin of d.rect development in the genera *Taylorana* and *Philautus*. *C r. Acad. Sci., Sci. Vie*, **323**: 287-297.

MENSI, P., LATTES, A , MARCARIO, B , SALVIDIO, S., GIACOMO, C & BALLETTO, E., 1992 – Taxonomy and evolution of European brown frogs. *Zool. J. Linn. Soc.*, **104**: 293-311

MILSTEAD, W. W., RAND, A. S. & STEWART, M M , 1974 – Polymorphism in cricket frogs: an hypothesis. *Evolution*, **28**: 489-491.

MOHANTY, A. K & DUTTA, S. K ,1999. – Biological notes on *Lunnonectes limnocharis* (Anura: Ranidae) in India. *Russ. J. Herp.*, **6**: 33-44

MORIWAKI, T , 1953 The inheritance of the dorso-median stripe in *Rana limnocharis* Wiegmann. *J. Sci Hiroshima Univ.*, (B), div.1, **14**: 159-164.

NEI, M., 1972. – Genetic distance between populations. *Am. Nat* , **106**: 283-293.

NISHIOKA, M. & SUMIDA, M., 1990 - Differentiation of *Rana limnocharis* and two allied species elucidated by electrophoretic analyses. *Lab. amphibian Biol , Hiroshima Univ* , **10**: 125 154

NORUSIS, M. J., 1992 *SPSS for Windows. Professional Statistics. Release 5* Chicago, SPSS Inc. [I-XII] + 1-348.

OHLER, A., 1996 Systematics, morphometrics and biogeography of the genus *Aubria* (Ranidae, Pyxicephalinae). *Alytes*, **13**: 141-166.

----- 1999 The identity of *Dendrobatorana* Ahl, 1927 (Amphibia, Ranoidea). *Mitt. Mus. Naturkd. Berlin*, **75**: 37-45.

OHLER, A. & DUBOIS, A., 1999. The identity of *Elachyglossa gyldenstolpei* Andersson, 1916 (Amphibia, Ranidae) with a discussion of some aspects of statistical support to taxonomy. *Zool. scripta*, **28** 269-279.

RICHARDSON, B. J., BAVERSTOCK P R. & ADAMS, M , 1986 *Allozyme electrophoresis. A handbook for animal systematics and population structure*. Sydney, Academic Press.

ROE, B. A , DIN-POW, M , WILSON, R K. & WONG, J. F , 1985. The complete nucleotide sequence of the *Xenopus laevis* mitochondrial genome. *J. biol. Chem.*, **260**: 9759-9774

ROHLF, F J., 1990 *NTSYS Numerical Taxonomy and Multivariate Analysis System, version 1.60* New York, Setauket.

SAMBROOK, J., FRITSCH, E F & MANIATIS, T., 1989. *Molecular cloning : a laboratory manual* Cold Spring Harbor Laboratory Press: 1-729.

SHIBATA, Y. 1988 Mid-dorsal stripe type of a frog, *Rana limnocharis*, in northwestern islands of Kyushu, Japan (Amphibia: Ranidae). *Shizenshi-Kenkyu occ. Pap. Osaka Mus. nat. Hist* , **2** (4) 69-71

SIGISMUND, H R , 1993 – *G-STAT, version 3*. Munich.

SWOFFORD, D. L , 1993 *PAUP Phylogenetic Analysis Using Parsimony, version 3.1.1* Computer program distributed by the Illinois Natural History Survey, Champaign, Illinois.

TODA, M , MATSUI, M , NISHIDA M & OTA H , 1998*a* Genetic divergence among Southeast and East Asian populations of *Rana limnocharis* (Amphibia: Anura), with special reference to sympatric cryptic species in Java. *Zool Sci* , **15**: 607-613.

TODA, M , NISHIDA, M , MATSUI, M , FU, W G & OTA, H , 1997 Allozyme variation among East Asian populations of the Indian rice frog, *Rana limnocharis* (Amphibia: Anura). *Biochem Syst Ecol* , **25**: 143-159

TODA, M NISHIDA, M MATSUI, M , LUI, K Y & OTA, H , 1998*b* Genetic variation in the Indian rice frog, *Rana limnocharis* (Amphibia: Anura), in Taiwan, as revealed by allozyme data. *Herpetologica*, **54** (1):73-82

VEITH M , 1994 Morphological, molecular and life history variation in *Salamandra salamandra* (L.) *In* H GREVEN & B THELSMEIER (ed), *Biology of Salamandra and* Mertensiella. *Mertensiella*, **4** 355-398

----- 1996 Molecular markers and species delimitation: examples from the European batrachofauna. *Amphibia-Reptilia*, **17**: 303-314

Corresponding editor: W. Ronald HEYER

Appendix 1 List of morphometric measurements of *Fejervarya* specimens used in this study.

Abbreviation	Measurement
EL	eye length
EN	distance from front of eye to nostril
FFTF	webbing measured from maximum incurvation between toes IV and V to tip of toe IV
FLL	length of arm from elbow to base of outer palmar tubercle
FOL	length of foot from proximal border of inner metatarsal tubercle to tip of toe IV
FTL	length of fourth toe from basal border of proximal subarticular tubercle to tip of toe IV
HAL	length of hand from base of outer palmar tubercle to tip of finger III
HL	head length from mandibular articulation to tip of snout
HW	head width
IBE	distance between posterior borders of eyes
IFE	distance between anterior borders of eyes
IMT	length of inner metatarsal tubercle
IN	internarial distance
ITL	inner toe length from distal border of inner metatarsal tubercle to tip of toe I
MBE	distance from mandibular articulation to posterior border of eye
MFE	distance from mandibular articulation to anterior border of eye
MN	distance from mandibular articulation to nostril
MTFF	webbing measured from distal border of inner metatarsal tubercle to maximum incurvation between toes IV and V
MTTF	webbing measured from distal border of inner metatarsal tubercle to maximum incurvation between toes III and IV
SVL	snout-vent length
TFL	length of finger from basal border of proximal subarticular tubercle to tip of finger III
TFTF	webbing measured from maximum incurvation between toes III and IV to tip of toe IV
TL	length of tibia from tibio-metatarsal articulation to knee
TYD	maximum tympanum diameter
TYE	tympanum-eye distance

Appendix 2 Allele frequencies, average expected heterozygosity (H_e) and polymorphism (P) at 15 presumptive enzyme loci in 9 samples of *Fejervarya* from Java (J), Kalimantan (K) and Sumatra (S). See text (*Material and methods*) for details on localities. Sample Kc belongs to the species *Fejervarya cancrivora*

Sample (n)	J1 (3)	J2 (11)	K1 (2)	K3 (12)	S1 (13)	S2 (2)	S5 (5)	S6 (3)	Kc (25)
ACON-1	a	b	a	a	a	a	a	a	a
ACON-2	b	a	b	b	b	b	b	b	c
FUM	a	a	a	a	a	a	a	a	a
AAT-1	c	a (0.05) c (0.90) d (0.05)	c	b (0.04) c (0.96)	c	c	c	c	e (0.02) f (0.98)
AAT-2	a	b	a (0.25) b (0.75)	a (0.70) b (0.30)	a	a	a	a	c
IDH-1	a	a	a	a	a	a	a	a	b
IDH-2	a	a	a	a	a	a	a	a	a (0.96) b (0.04)
LDH	a	b	a	a	a	a	a	a	c
MDH	a (0.87) b (0.13)	a	c	c (0.96) d (0.04)	b (0.09) c (0.50) d (0.41)	c (0.25) d (0.75)	d	c (0.50) d (0.50)	e
ME	a	a	a	a	a	a	a	a	b
MPI	d	b (0.59) c (0.41)	a (0.25) c (0.50) e (0.25)	a (0.25) b (0.45) d (0.25) e (0.05)	b (0.35) d (0.65)	b	b	b	b (0.60) f (0.14) g (0.26)
PEP (Gly-Leu)	b (0.37) c (0.63)	a	b	b	a (0.15) b (0.85)	b	b	b	b
PGM	a	a	a	a (0.84) b (0.16)	a (0.88) b (0.12)	a	a	a	c
PK	a	a	a	a	a	a	a	a	b
TPI	b (0.87) c (0.13)	b (0.95) c (0.05)	a (0.25) b (0.50) c (0.25)	a (0.05) b (0.95)	b	b	b	b	b
H_e	0.26	0.05	0.11	0.15	0.1	0.02	0	0.03	0.04
P	0.2	0.2	0.2	0.4	0.26	0.06	0	0.06	0.2

Appendix 3 – Morphometrical data for adult specimens of *Fejervarya* samples from Sunda Islands Mean, standard deviation, minimum and maximum values are given for all samples

Measurement or ratio	Chanjur (Java) 5 males	Medan (Sumatra) 2 males	Desa Seleukat (Sumatra) 1 male	Desa Lape (Kalimantan) 4 males	Bogor (Java) 1 female	Chanjur (Java) 1 female	Medan (Sumatra) 7 females
EL / SVL	106 ± 2 89 102 109	119 ± 10 37 112 127	129	119 ± 5 19 114 – 126	96	111	107 ± 6 03 97 115
EN / SVL	94 ± 4 75 88 – 99	98 ± 4 65 95 – 101	91	87 ± 6 82 – 95	86	87	92 ± 4 83 87 – 100
FLL / SVL	209 ± 6 12 199 – 215	222 ± 4 56 219 – 226	221	222 ± 5 52 218 230	221	2.1	2.9 ± 9 46 191 - 218
HDL / SVL	524 ± 27 16 489 557	543 ± 7 87 538 549	528	535 ± 15 47 522 - 557	496	5.8	524 ± 22 68 490 - 548
HTL / SVL	323 ± 13 44 308 337	319 ± 5 44 315 323	337	324 ± 7 37 315 - 333	303	320	309 ± 13 64 292 325
HAL / SVL	212 ± 8 74 200 224	192 ± 17 68 179 - 204	215	206 ± 15 82 186 - 223	176	200	195 ± 10.49 176 210
HL / SVL	395 ± 17 18 374 416	415 ± 45 75 383 - 447	399	388 ± 9.21 375 - 395	375	364	382 ± 5 85 374 - 389
HW / SVL	358 ± 12 337 – 367	351 ± 4 96 347 - 354	353	345 ± 6 21 340 - 354	348	342	350 ± 13 19 332 - 365
IBE / SVL	239 ± 5 54 232 – 245	235 ± 19 81 221 - 249	248	247 ± 18 43 231 – 273	210	239	224 ± 7 84 212 – 236
IUE / SVL	152 ± 10.67 141 – 162	157 ± 6.72 153 – 162	160	157 ± 9 77 144 – 168	129	145	148 ± 8 41 137 162
IMT / SVL	44 ± 3.19 43 – 48	51 ± 1 67 50 – 53	–	50 ± 3.56 46 – 53	56	44	53 ± 3.21 48 – 57
IN / SVL	74 ± 1 68 72 76	75 ± 1 96 73 76	72	78 ± 5 7 71 – 85	66	68	74 ± 2 87 70 – 79
ITL / SVL	110 ± 10.1 96 – 124	117 ± 5 35 113 – 120	119	119 ± 4 21 114 – 124	96	102	115 ± 5.64 103 122
IUE / SVL	54 ± 2 41 50 - 56	50 ± 6.09 46 - 55	52	50 ± 3 93 46 – 54	46	50	48 ± 3.51 40 – 51
MBE / SVL	163 ± 20 53 138 - 188	154 ± 24 04 137 171	147	154 ± 13 9 134 – 167	154	140	145 ± 7 23 138 159
MTL / SVL	255 ± 19 8 234 - 275	266 ± 40 71 237 – 294	26	249 ± 9 31 237 - 260	255	235	243 ± 9 28 232 254
MN / SVL	340 ± 19 07 318 360	360 ± 46 64 327 393	337	329 ± 9 97 320 – 341	326	309	323 ± 0 99 310 – 341
SVL	41 7 ± 1 14 40 4 – 42.7	39.3 ± 8.42 33 3 – 45 2	32 6	41 9 ± 3 53 38.1 – 46.7	55 2	45 6	54 7 ± 2.53 51 7 – 59 0
TFL / SVL	124 ± 11 21 1 1 136	120 ± 2 17 119 122	123	124 ± 6 23 118 132	114	124	114 ± 7 05 102 123
TL / SVL	494 ± 17 5 468 - 511	516 ± 2 96 514 – 518	494	507 ± 14 72 488 – 523	489	491	514 ± 20 85 475 – 537
TYD / SVL	67 ± 2 87 63 70	65 ± 4 57 62 68	60	67 ± 5 93 59 73	66	63	62 ± 4 1 58 67
TY / SVL	33 ± 2 93 3 - 38	35 ± 5 22 32 – 39	34	40 ± 3 64 37 43	38	4	37 ± 2 61 35 42
UEW / SVL	83 ± 5 98 76 88	89 ± 2 83 87 91	9.	91 ± 6 27 87 100	80	75	86 ± 3 06 82 – 92
WFE / SVL	81 ± 10.69 69 – 98	80 ± 3 05 77 – 82	91	78 ± 14 69 61 - 97	75	72	78 ± 8 36 63 85
WL / SVL	7(± 12 99 (4 96	83 ± 3 78 80 - 86	87	78 ± 9 91 69 – 92	75	87	7> ± 7 57 66 88
WUE / SVL	62 ± 12 11 47 80	56 ± 2 76 54 58	70	60 ± 13 86 46 79	59	55	5> ± 9 19 4(65
WTE / SVL	87 ± 12 03 76 106	94 ± 4 94 90 97	99	90 ± 6 79 82 – 98	86	95	85 ± 6 83 78 98

Appendix 3. – Continued

Measurement or ratio	Sidikalang (Sumatra) 1 female	Tapaktuan (Sumatra) 4 females	Desa Seleukat (Sumatra) 2 females	Desa Lape (Kalimantan) 8 females	Pontianak (Kalimantan) 2 females	Java *R. limnocharis* neotype 1 female
EL / SVL	104	116 ± 4 73 111 – 121	108 ± 1 04 107 – 109	113 ± 3 97 109 – 120	116 ± 2 7 114 – 118	117
EN / SVL	80	89 ± 4 04 85 – 93	89 ± 2 34 87 – 91	88 ± 3 93 81 – 91	94 ± 1 07 94 95	91
FLL / SVL	208	217 ± 5 77 212 – 225	224 ± 3 79 222 – 227	220 ± 7 79 207 – 230	225 ± 5 85 221 – 229	196
FOL / SVL	509	545 ± 18 31 519 563	548 ± 7 73 543 – 554	524 ± 28 13 465 – 558	547 ± 18 37 534 – 560	554
FTL / SVL	306	330 ± 10 13 315 – 338	332 ± 0 96 331 – 333	313 ± 18 46 273 – 337	336 ± 11 74 328 – 344	324
HAL / SVL	219	190 ± 14 08 173 – 203	203 ± 9 9 196 – 210	201 ± 11 87 180 – 221	208 ± 4 94 204 – 211	221
HL / SVL	380	391 ± 12 67 373 – 402	376 ± 9 9 369 – 383	393 ± 18 07 363 – 416	385 ± 3 82 382 388	329
HW / SVL	344	337 ± 4 11 332 – 342	352 ± 0 44 351 – 352	346 ± 16 27 319 – 367	359 ± 0 87 359 – 360	360
IBE / SVL	208	226 ± 4 59 221 231	214 ± 4 05 212 217	233 ± 12 84 217 251	241 ± 11 24 233 249	246
IFF / SVL	144	141 ± 6 62 135 – 148	139 ± 3.66 137 – 142	153 ± 9 5 141 – 169	167 ± 8 84 161 – 173	142
IMT / SVL	45	50 ± 2 86 47 – 53	51 ± 0 46 51 – 52	52 ± 3 94 47 – 57	53 ± 0 83 52 53	48
IN / SVL	75	72 ± 4 48 68 78	70 ± 1 75 69 – 71	73 ± 4 47 66 – 79	76 ± 0 12 76 – 76	69
ITL / SVL	113	116 ± 5 48 111 – 123	119 ± 5 82 115 – 123	118 ± 6 39 108 – 125	118 ± 5 53 114 – 122	125
IUE / SVL	45	53 ± 5 47 – 59	48 ± 2.32 47 – 50	49 ± 5 7 40 – 58	40 ± 0 17 40 – 40	50
MBE / SVL	151	150 ± 14 25 137 – 169	157 ± 12 44 148 – 166	164 ± 17 68 143 – 193	139 ± 4 75 136 42	140
MFE / SVL	226	254 ± 10 19 240 265	244 ± 2 26 242 246	255 ± 17 95 229 – 279	243 ± 5 3 239 247	221
MN / SVL	311	331 ± 10 09 317 – 339	323 ± 2 97 321 325	339 ± 19 96 304 373	333 ± 7 0 3 328 – 338	306
SVL	57 6	42 9 ± 555 36 4 49 8	51 1 ± 1 34 50 1 52 0	48 1 ± 6.86 36 2 – 54 2	46 8 ± 2 48 45 0 48 5	444
TTL / SVL	116	123 ± 7 07 114 130	120 ± 5 85 116 124	122 ± 9 53 105 – 135	125 ± 5 12 122 129	130
TL / SVL	500	515 ± 18 78 488 530	528 ± 1 34 527 529	508 ± 20 82 465 – 534	507 ± 2 64 505 509	532
TYD / SVL	63	64 ± 2 09 62 66	62 ± 3 75 60 65	65 ± 2 94 62 71	68 ± 5 56 64 72	66
TYT / SVL	43	39 ± 3 35 35 44	39 ± 2 83 37 41	39 ± 5 77 27 49	41 ± 3 69 39 – 44	35
ULW / SVL	83	86 ± 3 84 81 89	78 ± 1 16 77 – 79	90 ± 3 84 86 – 98	93 6 ± 0 0 93 6	88
WTF / SVL	68	73 ± 8 94 65 – 85	81 ± 1 22 80 – 82	76 ± 12 3 52 86	87 ± 4 63 84 91	102
WT / SVL	65	76 ± 4.88 72 – 82	83 ± 5 76 79 87	78 ± 8 84 63 90	88 ± 6 59 83 – 92	83
WH / SVL	50	58 ± 11 52 49 75	68 ± 0 89 67 – 69	58 ± 7 84 47 68	68 ± 3 6 65 – 71	79
WTE / SVL	74	85 ± 10 56 77 – 100	94 ± 0 68 93 – 94	88 ± 8 37 76 – 100	98 ± 8 13 92 – 104	98

Source - MNHN, Paris